

**Research Article****The Response of Foxtail Millet Candidate Varieties from Nagekeo Regency to Leaf Blight (*Bipolaris setariae*)****Suriani^{1*}, Septian Hary Kalqutny¹, Amran Muis¹**¹*Indonesian Cereals Research Institute, Jl. Dr. Ratulangi No. 274 Maros*

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ABSTRACT

Foxtail millet has the potential to be developed as a healthier food alternative because of its high nutritional value. Disease such as leaf blight caused by *Bipolaris setariae* is one of the limiting factors in Foxtail millet productivity. One of the efforts to control the pathogen is by utilizing resistant varieties. In this study, two candidate varieties and two germplasm accessions were tested to determine the level of resistance to *Bipolaris setariae* leaf blight. The study was arranged based on a complete randomized design with six replications. Each test material was inoculated with the spore suspension at 4 WAP. Disease intensity was observed based on the disease scoring at 7, 9, and 11 WAP. AUDPC value is calculated based on the intensity of the attack at a particular observation time. Grain weight was recorded and statistically analyzed. The two candidate varieties of foxtail millet Pagamogo and Tadamude from Nagekeo Regency showed a moderately resistant response to leaf blight and had the lowest AUDPC values of 907.69 and 912.31. The highest increase in AUDPC values was observed in the initial observation period at 0-49 DAP.

Keywords: AUDPC; *Bipolaris setariae*; Foxtail millet; Leaf blight; Plant resistance.

1. Introduction

Foxtail millet had been a staple food in some parts of the world before rice cultivation was widely known to the public (Rini 2018). In Indonesia, Foxtail millet is generally used as bird food. However, in some areas on Buru Island (Maluku) and Numfor Island (Papua), this crop is used as an alternative food (Randall *et al.* 2016). Foxtail millet can grow well in high temperature, limited water availability, and without fertilizers. It can even grow in suboptimum lands that are difficult to plant other food crops such as wheat and rice (Bhuja 2009). Saxena *et al.* (2018) reported that millets have a good adaptation in areas with low rainfall.

Foxtail millet contains several nutrients, including protein, minerals, and vitamins which are typically higher than rice and wheat. The average protein content of some foxtail millet germplasm from India is 12.63% (Kamatar *et al.* 2015). Foxtail millet also has lower carbohydrate content than rice and wheat. Foods with low carbohydrate and high fiber content are very good to be used as healthier alternative food, especially for diabetics and heart

disease patients (Kamatar *et al.* 2015). This potential can be optimized by making the foxtail millet as local food to promote food diversification, thus reducing rice and imported food (Randall *et al.* 2016).

Because of the potential of foxtail millet as healthier alternative food, foxtail millet development through breeding were carried out to improve or obtain the desired unique characteristics from the existing germplasm collection. East Nusa Tenggara Province is known to be an area with a high number of foxtail millet germplasm. Therefore, the Nagekeo Regency government, in collaboration with the Assessment Institute for Agricultural Technology of NTT, develop the local germplasms to become a newly released variety.

One of the important main characters in developing new varieties is the plant's resistance against pests and diseases. Some of the pests attacking foxtail millet are stem borers, birds, and leaf borers (Kalaisekar & Padmaja, 2017). The diseases that commonly occur in foxtail millet are blast (*Pyricularia setariae*), brown leaf spot

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(*Drechslera setariae*), rust (*Uromyces setariae*), downy mildew (*Sclerospora graminicola*) and soot (*Ustilago crameri*) (Konda 2015). Brown leaf spot disease or millet leaf blight caused by the fungi *Drechslera setaria* (Saw.) Subram. & Jain synonym of *Helminthosporium drechs*, *Bipolaris setariae* (Saw.) Shoem (Kumar 2011; Kadam & Sharma 2018; Manamgoda *et al.* 2014). *B. setariae* is also known to have a broad host range, Niu *et al.* (2014) reported that the pathogen also attacks the seeds of coconut plants. In a separate study, Duan *et al.* (2012) also reported a *B. setariae* attack on cassava. The study conducted by Kadam & Sharma (2018) reported that the main diseases of millet during 2017 in India with the highest disease severity are leaf blight and leaf rust. Furthermore, Timilsina *et al.* (2016) also reported that leaf blight is a major disease of millet and the tests conducted showed that all 12 accessions of millet germplasm collected from several regions in Nepal were affected by leaf blight. The yield losses caused by leaf blight caused by *Bipolaris setariae* on foxtail millet is remain to be determined.

Leaf blight on millet can cause damage in all stages of plant growth, from seed to seed formation (Kumar 2011). Symptoms are characterized by the formation of lesions on the leaves; over time, the lesions fuse and can cause the dry leaf. Diseases that attack the leaves can cause a decrease in yield because plants experience a decrease in photosynthetic areas, increased respiration rates, and there is a decrease in photosynthate translocation from infected tissue (Binghan *et al.* 2009). Early infection in millet plants can cause seedling rot (Das *et al.* 2016). *Bipolaris setariae* can be transmitted through seeds or survived on infected plants residues on the field as a source of inoculums (Das *et al.* 2016; Nasnwa *et al.* 2018). Leaf blight on foxtail millet that can cause economic losses have never been reported in Indonesia, but in 2008 it was reported a severe and widespread attack in Iran's South Khorasa region (Mirzaee *et al.* 2010).

Plant diseases control by using resistant varieties is considered effective because it can prevent the spread of the disease as demonstrated by other cereal crops (Altaf *et al.* 2016; Al-Saidi 2016). However there are no report regarding the use of resistance varieties to control tis disease. Disease-resistant varieties are developed by a series of breeding and evaluation. This study was conducted to evaluate two candidate varieties of foxtail millet from Nagekeo Regency against leaf blight. This candidate varieties are expected to have resistance to blight so that they become a new superior variety that will be developed.

2. Materials and Methods

The study was carried out in the Cereals Plant Research Institute greenhouse in August to November 2018. A total of four accessions were used in this study, namely 1-A, 1-B, 8, and 12-B. Accessions 1-A and 1-B are candidate varieties of foxtail millet from the Nagekeo Regency in East Nusa Tenggara, while accessions 8 and 12-B are two of the germplasm collections of ICERI (Indonesian Cereals Research Institute). The research was arranged based on a Complete Randomized Design with six replicates.

Bipolaris setariae isolates preparation

Infected leaves were collected and isolated in the laboratory by growing the pathogen on Potato Dextrose Agar (PDA), and incubated for two days. Fungal hyphae that grow were purified and incubated for 14 days in an incubator. The fungi were identified microscopically and macroscopically by looking at the morphological form of the fungus. The isolates were later grown on PDA and incubated for 12 days as inoculant material.

Field Inoculation

The foxtail millet were planted in a plot with an area of 1 × 5 m, with the spacing of 75 × 20 cm, 2 rows each. Seeds are planted directly by making holes in the ground. Maintenance includes weeding, fertilizing and watering. Fertilization was done based on Tatuhey (2014) recommendation, namely urea 200 kg ha⁻¹, SP-36 83 kg ha⁻¹, and KCL 100 kg ha⁻¹ on 10 and 30 days after planting (DAP). Artificial pathogen inoculation was carried out at four weeks after planting (WAP) by spraying the plant materials with the suspension of pathogen spores that had been prepared in the laboratory with a density of 10⁶ spores/ml in the afternoon as much as approximately 10 ml/plant. Before the plants are inoculated with pathogens, watering is done to increase plant moisture. The research were conducted in an isolated screening house to minimize the spread of the disesase outside the research area.

The observation of the severity of leaf blight

The severity of the disease was first observed 3 weeks after the inoculation of the pathogen and was observed every 2 weeks by scoring 10 random samples per row. The scoring value is then transformed to the severity of the disease by using the following formula:

$$DS = \frac{\sum(n_i \times v_i)}{Z \times N} \times 100\%$$

where, DS=disease severity; n =infected plant number;
v =score ; Z = highest score used (5); N =
number of plants observed

Disease severity scores namely 0 = no symptoms; 1 = spots of more than 1% of the leaf surface; 2 = 5-10 % on the leaf surface; 3 = brown spots with gray center around 5-25% of the leave surface; 4 = 25-50% discoloration of adjacent segments; 5 = More than 50% of infected leaves.

The determination of the level of plant resistance to leaf blight disease was categorized by using the following criteria, namely 0 - 5%: Highly Resistant; 5-20%: Resistant; 20-40%: Moderately Resistant; 40-60%: Susceptible; and > 60%: Highly Susceptible.

To determine the disease's progress in each material tested, data of the disease severity were plotted against the time of observation to get the disease development curve. For statistical analysis, the area under the disease development curve (Area Under Diseases Progress Curve, AUDPC) is calculated using the following formula (Mehmood *et al.* 2016):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

where, X=disease intensity; t= time of observations; n= number of observations

Grain weight

The total weight of the grains from two rows of plants in each unit was weighed. The data for each parameter were analyzed by using the STAR Ver. 2.0.1 for Windows. The data were analyzed statistically by using ANOVA followed by Duncan post-hoc test.

3. Results

The severity of the leaf blight

Based on the observations, the symptoms of leaf blight caused by *B. setariae* on foxtail millet began to be found in the vegetative phase with the appearance of elongated lesions on the lower leaves (Figure 1a). Symptoms then spread to the top leaves. The lesions formed were brown with pronounced borders. The lesions develop and fused so that the infected leaves become dry. The microscopic morphology of *B. setariae* conidia was straight ellipse with both ends slightly bent and have septae (Figure 1b).

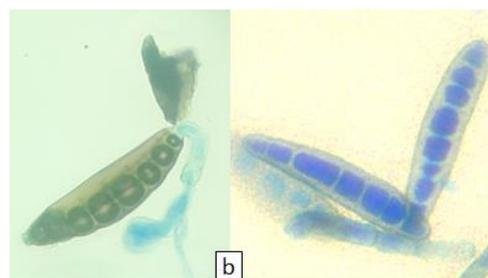


Figure 1. Symptoms of foxtail millet leaf blight (a); Conidia of *B. setariae* (b)

The severity of leaf blight disease observed on the test materials at 7 WAP was began to vary and significantly different statistically. Candidate varieties Pagamogo and Tedamude showed a low attack percentage and were significantly different with genotype 12-B that was infected by 30% (Figure 2). At the age of 9 WAP, the results did not show any significant difference between two candidate varieties and genotype 8. The genotype 12-B that had the highest disease at the beginning of the observation showed the highest disease severity of 49% and continued to increase until reaching 58% at 11 WAP and significantly different from the other 3 genotypes.

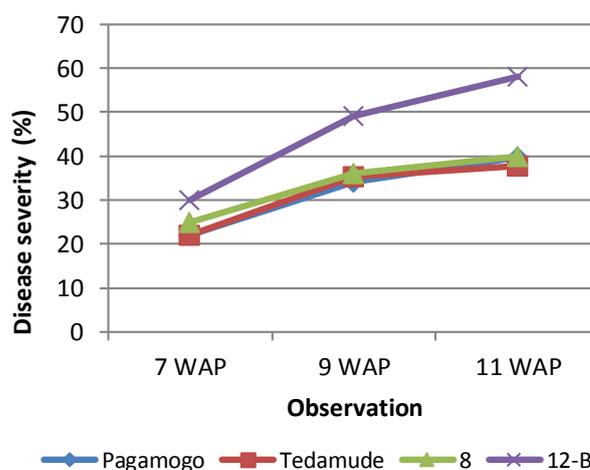


Figure 2. The disease severity at 7, 9 and 11 WAP in Maros, 2018.

The resistance of the foxtail millet against leaf blight disease

The results of the disease severity at 11 WAP showed that there were 3 genotypes of foxtail millet that were infected less than 40% severity or can be categorized as moderately resistant to leaf blight, namely genotype Pagamogo, Tedamude and 8 (Table 1).

Table 1. Resistance levels of four foxtail millet genotypes to leaf blight in Maros, 2018.

Genotypes	The average of the disease at 11 WAP (%)	Resistance Category
Pagamogo	39.67b	Moderately Resistance
Tedamude	37.67b	Moderately Resistance
8	40.00b	Moderately Resistance
12-B	58.00a	Susceptible

Notes: Different letters indicate significant differences between genotypes at α 0.05.

Based on the AUDPC value calculation, the two candidate varieties of foxtail millet from Nagekeo Regency showed the lowest values of all tested materials, 907.69 and 912.31, respectively. In comparison, genotype 12-B, which is categorized as susceptible to millet leaf blight, showed an AUDPC value of 1,302 (Table 2).

Table 2. Average values of AUDPC of leaf blight in foxtail millet genotypes in Maros, 2018.

Genotypes	AUDPC
Pagamogo	907.69
Tedamude	912.31
8	959
12-B	1,302

The increase in the value of the AUDPC in the particular observation period can be seen in Figure 3. The highest increase of AUDPC value was observed in the initial observation period, which is 0-49 DAP, then followed by the 49-63 DAP period. The lowest increase of AUDPC value was observed in the 63-77 DAP period; this shows that the highest increase in attack intensity was found in the initial growth period.

Difference in AUDPC value at T

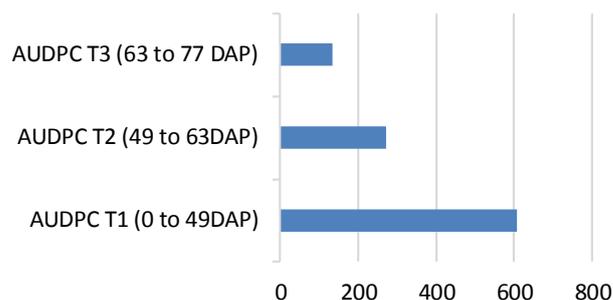


Figure 3. The increase of the AUDPC value in a particular time span of observation (0-49 DAP, 49-63 DAP, 63-77 DAP).

Grain Weight

The average grain weight of each test material showed statistically significant differences between accessions. Accessions 1-B produced the highest grain weight (520.87 g) and significantly different from the other three accessions (Table 3). While the 12-B genotype that showed the highest percentage of disease severity results have the lowest grain weight (237.28 g) compared to other accessions

Table 3. Average grain weight and harvest time of 4 foxtail millet Genotypes in Maros, 2018.

Genotypes	Grain weight (g)	Harvest Time (DAP)
Tedamude	520.87 a	84
Pagamogo	363.812 b	89
8	245.40 b	88
12-B	237.28 b	76

Notes: Different letters indicate significant differences between genotypes at α 0.05.

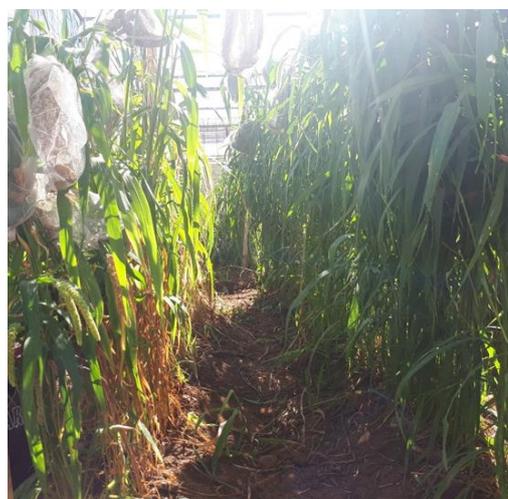


Figure 4. The disease severity comparison between Susceptible variety (left) and Moderately Resistance variety (right).

4. Discussion

The symptoms of the disease observed in this study were similar symptoms by the reported by Kumar (2011) which stated that *B. setariae* caused elongated spots with irregular shapes, sizes varying from 3 to 20 mm, dark brown with lighter border color and sometimes causing the veins of the leaf broken. The symptoms of disease infection appeared 7-10 days after inoculation of the pathogen.

The high severity of blight can affect plant development because it disturbs photosynthesis processes. The leaf blight causes elongated brownish lesions on the leaves. Lesions formed will reduce the photosynthetic area and indirectly reduce production (Khaeruni *et al.* 2014). The lesions will fuse so that the leaves will dry and can even kill the plant. This will disturb the process of plant metabolism. Yield losses due to leaf blight attacks depend on the variety planted, the age of the plant infected, environmental conditions, and geographical areas (Kumar 2011). The change in temperature trends can affect the disease equilibrium in some region, the importance of major disease may reduce, and vice versa the importance of the minor disease increase (Karkee *et al.* 2020)

Genotype 12-B was infected up to 58% and categorized as Susceptible. The genotype that showed consistently high levels of resistance could be further developed to be a released variety. The genotype that showed good resistance to the disease until 11 MST have fewer yield losses due to the disease because it has completed the seed filling stage and entered the seed maturation stage.

The two candidate varieties of foxtail millet from Nagekeo Regency showed the lowest values of all tested materials, 907.69 and 912.31, respectively. In comparison, genotype 12-B, categorized as susceptible to millet leaf blight, showed an AUDPC value of 1,302. Genotype with low AUDPC values indicate the suppression of disease progression due to the mechanism of resistance inherited by these plants.

The lowest increase of AUDPC value in 63-77 DAP period indicate that the highest increase in attack intensity was found in the initial growth period. The initial period of growth becomes a critical susceptible period for foxtail millet to *B. setariae* infection. Plants do not have known immune systems like in animals, but they have developed wide defences mechanisms such as structural, chemical, and protein-based defences (Freeman & Beattie, 2008). The plant defense happens before (pre-existing barriers) and during pathogen infection (pathogen- or microbe-

associated molecular patterns/ PAMPs or MAMPs) (Garcion *et al.* 2014). Furthermore, Zhinhuan *et al.* (2000) reported that the plants resistant to blight generally involve the production of enzymes in cell walls that can suppress the pathogens' growth.

Besides, the resistance might also involve the ability of the plant to form a specific structure that can inhibit the development of pathogens, this structural changes such as the lack of stomata per unit area of the leaf, thickness of the cuticle layer, the formation of tissue of cork, or the production of toxic substances before or after the pathogens entered the plant tissue, so the pathogens fail to develop further and cause disease (Andersen *et al.*, 2018). Pathogens that successfully infect tissue can cause damage to epidermal cells, the nucleus of the cells and other organelles, ultimately causing severe damage to the leaves (Dalimunthe *et al.* 2015).

The low grain weight on the varieties with the highest disease severity study suggested that this disease may affect production. Ramappa *et al.* (2006) reported that leaf blight can reduce the weight of pearl millet grains up to 77% and the weight reduction varies between the varieties tested. The difference in weight of foxtail millet grains is influenced by disease attack and genetic differences in each plant.

5. Conclusion

Based on the study results, the two candidate varieties of foxtail millet 1 Pagamogo and Tedamude from Nagekeo Regency showed a moderately resistant response to leaf blight. They had the lowest AUDPC values of 907.69 and 912.31. Candidate variety Tedamude had the highest grain weight compared to other genotypes. The highest increase in AUDPC values was observed in the initial observation period at 0-49 DAP.

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7. Declaration of Conflicting Interests

The authors have declared no potential conflicts of interest concerning the study, authorship, and/or publication of this article.

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